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Alendronate decreases urine calcium and supersaturation in genetic hypercalciuric rats

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Background. The mechanism of excess urine calcium excretion in human idiopathic hypercalciuria (IH) has not been determined but may be secondary to enhanced intestinal calcium absorption, decreased renal calcium reabsorption, and/or enhanced bone demineralization. We have developed a strain of genetic hypercalciuric stone-forming (GHS) rats as an animal model of human IH. When these GHS rats are placed on a low-calcium diet (LCD), urinary calcium (U_{Ca}) excretion exceeds dietary calcium intake, suggesting that bone may contribute to the excess U_{Ca} excretion. We used the GHS rats to test the hypothesis that bone contributes to the persistent IH when they are fed an LCD by determining if alendronate (Aln), which inhibits bone resorption, would decrease U_{Ca} excretion.

Methods. GHS rats ($N = 16$) and the parent strain (Ctl, $N = 16$) were fed 13 g/day of a normal (1.2%) calcium diet (NCD) for seven days and were then switched to a LCD (0.02%) for seven days. Ctl and GHS rats in each group were then continued on LCD for an additional seven days, with or without injection of Aln (50 μg/kg/24 hrs). U_{Ca} excretion was measured daily during the last five days of each seven-day period. To determine the effects of Aln on urine supersaturation, the experiment was repeated. All relevant ions were measured, and supersaturation with respect to calcium oxalate and calcium hydrogen phosphate was determined at the end of each period.

Results. U_{Ca} was greater in GHS than in Ctl on NCD (7.4 ± 0.5 mg/24 hrs vs. 1.2 ± 0.1, GHS vs. Ctl, $P < 0.01$) and on LCD (3.9 ± 0.2 mg/24 hrs vs. 0.7 ± 0.1, GHS vs. Ctl, $P < 0.01$). LCD provides 2.6 mg of calcium/24 hrs, indicating that GHS rats are excreting more calcium than they are consuming. On LCD, Aln caused a significant decrease in U_{Ca} in GHS rats and brought GHS U_{Ca} well below calcium intake. Aln caused a marked decrease in calcium oxalate and calcium hydrogen phosphate supersaturation.

Conclusion. Thus, on a LCD, there is a significant contribution of bone calcium to the increased U_{Ca} in this model of IH. Aln is effective in decreasing both U_{Ca} and supersaturation. The Aln-induced decrease in urine supersaturation should be beneficial in preventing stone formation in humans, if these results, observed in a short-term study using the hypercalciuric stone-forming rat can be confirmed in longer term human studies.

Excess urine calcium (U_{Ca}) excretion of unknown etiology, so-called idiopathic hypercalciuria (IH), is common in patients with calcium nephrolithiasis and contributes to U_{Ca} oxalate supersaturation and stone formation [1–3]. There are several mechanisms, alone or in combination, that could be responsible for the increased U_{Ca} excretion: (*a*) increased intestinal calcium absorption, either directly or through an increase in the effect of $1,25(OH)_2D_3$, (*b*) decreased renal mineral reabsorption of either calcium or phosphorus, and/or (*c*) enhanced bone resorption [1, 4, 5].

Hypercalciuria appears spontaneously among rats [6], and mating of spontaneously hypercalciuric rats results in a successive increase of the IH among their offspring [7–18]. To help understand the mechanism(s) responsible for IH in humans, we have established an animal model of IH by inbreeding the most hypercalciuric progeny of spontaneously hypercalciuric male and female Sprague-Dawley rats [7–16]. Through over 46 generations of successive inbreeding, we have established a strain of rats, each of which excrete abnormally large amounts of U_{Ca}. The principal mechanism for the excessive calcium excretion in these rats appears to be an increase in intestinal calcium absorption [10, 14–16]. The increased calcium absorption appears to be mediated not by an increase in the serum level of $1,25(OH)_2D_3$, but by an increase in the number of intestinal vitamin D receptors [15]. When these hypercalciuric rats are fed a very low-calcium diet (LCD), their U_{Ca} excretion decreases, but remains elevated compared with that of similarly treated control (Ctl) rats, indicating an additional defect in renal calcium reabsorption and/or an increase in bone resorption [14]. Many of these hypercalciuric rats excrete more calcium than they consume, which is termed a negative calcium balance [14]. Because the majority of body calcium is contained within the bone mineral, the source of the additional U_{Ca} may be the mineral phases of bone [19].

The bone from these hypercalciuric rats releases more calcium, compared with bone from Ctl rats, when exposed to increasing amounts of $1,25(OH)_2D_3$ [11]. In addition, a primary defect in renal calcium reabsorption is observed during carefully controlled clearance studies [9]. We

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have shown that both the bone and kidney of the hypercalciuric rats also have an increased number of vitamin D receptors [11, 15]. Thus, these hypercalciuric rats appear to have a systemic abnormality in calcium homeostasis: they absorb more intestinal calcium; they resorb more bone; and they fail to reabsorb filtered calcium adequately. Because each of these hypercalciuric rats forms renal stones, we have termed the rats genetic hypercalciuric stone-forming (GHS) rats [7–10, 12]. Calcium transport abnormalities similar to those documented in the GHS rats have been observed in patients with IH [2].

In this study, we used the GHS rats to directly test the hypothesis that bone contributes to the persistent IH when these rats are fed a LCD and to determine if the bone resorption blocker alendronate (Aln) would alter U_{Ca} excretion and urine supersaturation with respect to the solid phases calcium hydrogen phosphate (brushite) and calcium oxalate (CaOx). We found that on a LCD there is a significant contribution of bone calcium to the increased U_{Ca} excretion and that Aln is effective in decreasing both U_{Ca} excretion and supersaturation. The Aln-induced decrease in urine supersaturation should be beneficial in preventing stone formation in humans, if these results, observed in the hypercalciuric stone-forming rat, can be applied to humans.

METHODS

Establishment of hypercalciuric rats

Initially, 20 male and 20 female adult Sprague-Dawley rats (Charles River Laboratories, Kingston, NY, USA) were screened for IH. The rats were placed in individual metabolic cages and were allowed five days to adjust to the cage and diet. During this time, the animals were fed 13 g/day of a diet containing 1.2% calcium, 0.90% phosphorus, 0.24% magnesium, 0.40% sodium, 0.43% potassium, and 2.2 IU vitamin D_3/gram of food. Deionized distilled water was provided *ad libitum.* Two successive 24-hour urine collections in 0.25 ml 12 N HCl were then obtained on days 6 and 7 to measure U_{Ca} excretion. The three male and three female rats with the greatest calcium excretion were used to breed the next generation. A similar protocol was used to select two to three of the most hypercalciuric males and three to four of the most hypercalciuric females for inbreeding of subsequent generations [7–16].

General study protocol

Sixteen female GHS rats and 16 female Sprague-Dawley Ctl rats, each weighing 150 g, were kept in metabolic cages for an additional three weeks. During the first week, all rats were fed 13 g/day of a normal calcium diet (NCD) containing 1.2% calcium, 0.65% phosphorus, 0.24% magnesium, 0.40% sodium, 0.43 potassium, and 2.2 IU vitamin D_3/g of food. During weeks 2 and 3, the rats received a diet that contained 0.02% calcium, which was matched to the 1.2% calcium diet. During week 3, one half of the GHS rats and one half of the Ctl rats, chosen at random, were given a subcutaneous injection of Aln 12.5 g/rat/day (approximately 50 μg kg/day) dissolved in 100 μl of deionized distilled water. This dose of Aln was found to inhibit bone resorption in rats significantly [20, 21]. The other half of the GHS and Ctl rats received an injection of only deionized distilled water. Deionized distilled drinking water was provided *ad libitum* throughout the course of each study.

Urinary calcium excretion study protocol

The GHS rats used for this study were from the 42nd generation. On days 2 through 7 of each week (week 1, week 2, and week 3), successive 24-hour urine collections in 0.25 ml 12 N HCl were obtained to measure U_{Ca} excretion. The metabolic cages were washed with 1.2 N HCl between collections to ensure that there was no residual calcium adhering to the stainless steel cages. All urine samples were refrigerated and measured for calcium within 48 hours.

Urine supersaturation study protocol

The GHS rats used for the second study were from the 46th generation. On days 6 and 7 of each week (week 1, week 2, and week 3) consecutive 24-hour urine collections were obtained. The first collection was in concentrated HCl and was used to measure oxalate and citrate. The second collection was in thymol and was used for all other determinations [7]. Again, the metabolic cages were washed with 1.2 N HCl between collections in order to ensure that there was no residual material adhering to the stainless steel cages. All urine samples were stored at −4°C, and all measurements were made within two weeks.

Any rat that ate less than 12 g of food or drank less than 15 ml of water on any day of either study would have been excluded from the entire study; however, all rats met this prospective criteria throughout the study.

Chemical determinations

Calcium was determined by reaction with arsenazo III and was determined photometrically [22]. Creatinine was determined by a modification of the Jaffe method [23]. Inorganic phosphorus was determined by reaction with ammonium molybdate to form a colored phosphomolybdate complex [24]. Uric acid was measured after oxidation by uricase to produce allantoin and hydrogen peroxide. The hydrogen peroxide reacts with 4-amino-antipyrine and 3,5-dichloro-2-hydroxybenzene sulfonate in a reaction catalyzed by peroxidase to produce a quinoneimine and was then determined photometrically [25]. Magnesium was determined by combination with calmagite and was measured photometrically [26]. Ammonia was deter-

mined by coupled enzyme system using glutamate dehydrogenase and nicotinamide adenine dinucleotide phosphate (NADPH) [27]. Sodium was determined by a selective electrode [28] and potassium using a valinomycin membrane attached to a potassium electrode [29]. Chloride was measured coulombmetrically [30]. Oxalic acid was measured utilizing oxalate oxidase, which oxidizes oxalate to hydrogen peroxide and carbon dioxide. The hydrogen peroxide then reacts with 3-methly-2-benzothiazolinone hydrozone and 3-(dimethyl)benzoic acid to form an indamine dye that is monitored photometrically [31]. Citric acid was determined using citrate lyase, which catalyzes the conversion citrate to oxaloacetic acid and which is then converted to malic acid, in the presence of malate dehydrogenase. The malic acid oxidizes NADH to NAD^+, which is monitored photometrically [32]. Sulfate concentration was measured by barium precipitation, monitoring turbidity photometrically [33]. pH was measured by an ion-selective electrode.

Urine supersaturation

The CaOx ion activity product was calculated using the computer program EQUIL developed by Finlayson and others [34–36]. The computer program calculates free ion concentrations using the concentrations of measured ligands and known stability constants. Ion activity coefficients are calculated from ionic strength using the Davies modification of the Debye-Huckel solution to the Poisson-Boltzman equation. The program simultaneously solves for all known binding interactions among the measured substances. Oxalate, phosphorus, and calcium ion activities were used to calculate the free-ion activity products. The free ions in solution are considered to be in an equilibrium with the dissolved CaOx governed by a stability constant (K) of 2.746×10^3 M^{-1} and with the dissolved brushite governed by a K of 0.685×10^3 M^{-1}. The value of CaOx in a solution at equilibrium with a solid phase of CaOx, the solubility of CaOx, is 6.16×10^{-6} M per liter. The value of the brushite in a solution at equilibrium with a solid phase of brushite, the solubility of brushite, is 3.981×10^{-7} M per liter. The relative supersaturation for CaOx is calculated as the ratio of the free-ion activity product of calcium and oxalate in the individual urine to the solubility of CaOx. The relative supersaturation for brushite is calculated as the ratio of the free-ion activity product of calcium and phosphate in the individual urine to the solubility of calcium phosphate. Ratios of one connote a sample at equilibrium, above one supersaturation, and below one undersaturation.

The ability of this computer program to predict accurately the saturation of urine or other solution with respect to the solid phase is excellent [7, 12–14, 34, 37–39]. With a series of 20 artificial solutions, the equilibrium calcium concentration and the extent of calcium precipitation were predicted with average errors of 5 ± 9% and 5 ± 8% (mean ± SD), respectively [34]. We have used this computer program previously and found excellent correspondence between calculated and experimentally measured saturations in urine and blood [7, 12–14, 38] and in bone culture medium [37, 39].

Statistical analysis

All values are expressed as mean ± SE. Tests of significance were calculated by analysis of variance with the Bonferroni correction for multiple comparisons using conventional computer programs (BMDP; University of California, Los Angeles, CA, USA) on a digital computer. P of less than 0.05 was considered significant.

RESULTS

Urinary calcium excretion study

During the first week, while eating a normal calcium diet (NCD) (1.2% Ca), the U_{Ca} excretion of the GHS rats was greater than that of the Ctl rats on each of the five days that urine was collected ($P < 0.001$, GHS vs. Ctl on each day, $N = 16$ in each group; Fig. 1). During the second week, while eating a low calcium diet (LCD; 0.02% Ca), the U_{Ca} of the GHS rats was again significantly greater than that of Ctl rats on each of the five days that urine was collected ($P < 0.001$, GHS vs. Ctl on each day, $N = 16$ in each group). During the third week, while eating LCD, the administration of Aln (approximately 50 μg/kg/24 hrs) led to a marked decrease in U_{Ca} in the GHS, but not in the Ctl, rats on each of the five days that urine was collected ($P < 0.001$, GHS vs. GHS + Aln on each day, $N = 8$ in each group).

Urine supersaturation study

Urinary calcium, magnesium, and phosphorus excretion. As in the U_{Ca} excretion study, during the first week of the urine supersaturation study, while eating NCD, U_{Ca} of the GHS rats was greater than that of Ctl rats ($P < 0.001$, GHS vs. Ctl, $N = 16$ in each group; Fig. 2). Over the second week, while eating LCD, the U_{Ca} of the GHS rats was far greater than that of Ctl rats ($P < 0.001$, GHS vs. Ctl, $N = 16$ in each group). Over the third week, while eating LCD, Aln (approximately 50 μg/kg/24 hr) led to a significant decrease in UCa in the GHS and in the Ctl rats ($P < 0.001$, GHS vs. GHS + Aln; $P < 0.05$, Ctl vs. Ctl + Aln, $N = 8$ in each group). Urine magnesium was slightly, but significantly, greater in the Ctl than in the GHS rats only during week 1 ($P < 0.05$). There was no difference in magnesium excretion between any other group throughout the remainder of the study, nor was urine Mg excretion affected by Aln in Ctl or GHS rats. During the first week, while rats were eating a NCD, there was no difference in phosphorus excretion between the Ctl and GHS rats; however, urine phosphorus excretion increased in all groups of rats fed

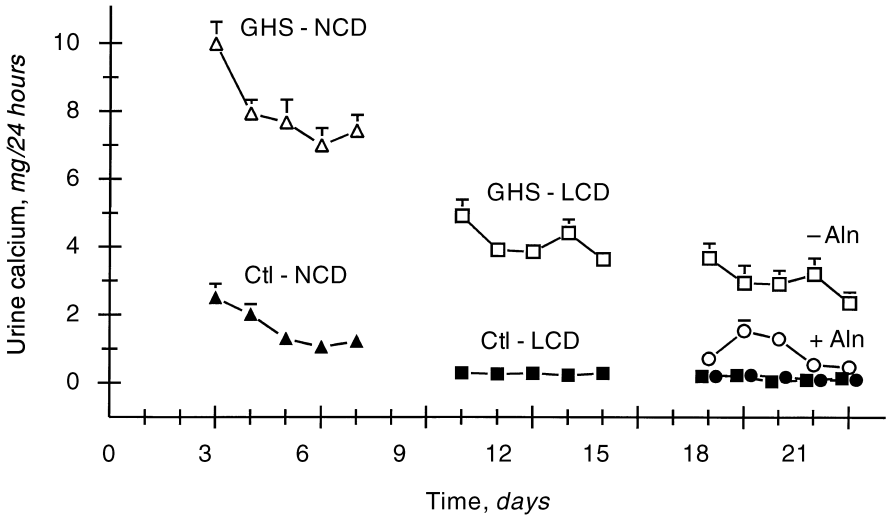

Fig. 1. Urine calcium excretion study. Daily urine calcium excretion in genetic hypercalciuric stone forming (GHS) and control (Ctl) rats. During all days of all three weeks, urinary calcium excretion (U_{Ca}) of GHS rats exceeded Ctl rats on each day ($N = 16$ in each group during weeks 1 and 2, $N = 8$ in each group during week 3). Aln led to a significant decrease in U_{Ca} in the GHS, but not in the Ctl, rats ($N = 8$ in each group). Abbreviations and symbols are: GHS, genetic hypercalciuric stone forming rats (open symbols); Ctl, control rats (closed symbols); NCD, normal calcium diet (1.2% calcium, triangles); LCD, low calcium diet (0.02% calcium, squares); LCD + Aln, alendronate (~Aln, 50 g/kg/24 hr, circles).

LCD during week 2 and week 3. There was no difference in urine phosphorus excretion in any group of rats during weeks 2 or 3, nor was urine phosphorus excretion affected by Aln in Ctl or GHS rats.

Urine oxalate and citrate excretion and pH. Urine oxalate excretion was greater in the Ctl than in the GHS rats during week 1 ($P < 0.05$; Fig. 3). There was no difference in oxalate excretion between any other group throughout the remainder of the study, and urine oxalate excretion was not affected by Aln. During week 1, there was no difference in urine citrate excretion between the GHS and the Ctl rats; however, urine citrate excretion was greater in each group of GHS rats compared with each group of Ctl rats during week 2 and week 3. Citrate excretion was not affected by Aln. During the entire study, the urine pH of each group of GHS rats was less then the urine pH of each group of Ctl rats during comparable weeks, and urine pH was not affected by Aln.

Urine ammonium and creatinine excretion and volume. During week 1, there was no difference in urine ammonium excretion between the GHS and the Ctl rats; however, urine ammonium excretion was greater in each group of GHS rats, compared with each group of Ctl rats during weeks 2 and 3 (Fig. 4). Ammonium excretion was not affected by Aln. There was no difference in urine creatinine or volume in any group during any period of the study. There was no difference in urine sodium, potassium, or chloride in any group during any period of the study.

Brushite and CaOx supersaturation. During the first week, when rats were fed a NCD, urine supersaturation with respect to both brushite ($CaHPO_4$) and CaOx was greater in the GHS rats than in Ctl rats (each $P < 0.001$, GHS vs. Ctl; Fig. 5). During the second week, when rats were fed LCD, urine supersaturation with respect to both brushite and CaOx in the GHS continued to exceed that in the Ctl rats (each $P < 0.001$, GHS vs. Ctl). During the third week, while eating LCD, Aln led to a marked decrease in both brushite and CaOx supersaturation in the GHS, but not in the Ctl, rats (each $P < 0.001$, GHS vs. Ctl). With Aln treatment, supersaturation with respect to brushite and CaOx in the GHS rats was not different from that of Ctl rats.

DISCUSSION

The inbred GHS rats have a marked increase in intestinal calcium absorption accounting for a significant degree of their IH [10, 14–16]. However, when these rats are placed on a LCD, U_{Ca} excretion continues to exceed that of Ctl rats and of dietary calcium intake [14]. Because bone contains the majority of total body calcium [40], the source of this additional U_{Ca} appears to be the mineral phases of bone. To directly test the hypothesis that bone allows the IH to persist when the GHS rats are fed a LCD, we used the bone-resorption blocker Aln [20, 21]. We found that Aln markedly decreases U_{Ca} excretion, demonstrating, unequivocally and to our knowledge for the first time, that bone mineral is the source of the additional U_{Ca} when the GHS rats are fed a LCD.

Supporting a primary role for enhanced bone resorption in maintaining the IH in the GHS rats consuming a LCD is the observation that cultured bone from these rats releases more calcium in response to $1,25(OH)_2D_3$, but not parathyroid hormone (PTH), than bone from Ctl rats [11]. We have previously shown that there are an increased number of vitamin D receptors in the bone from the GHS rats [11]. A LCD leads to an increase in $1,25(OH)_2D_3$ in normal [4, 41] and GHS [14] rats. Perhaps the increase in vitamin D receptor number results in a greater resorptive effect of the increased $1,25(OH)_2D_3$ on bone from the GHS rats.

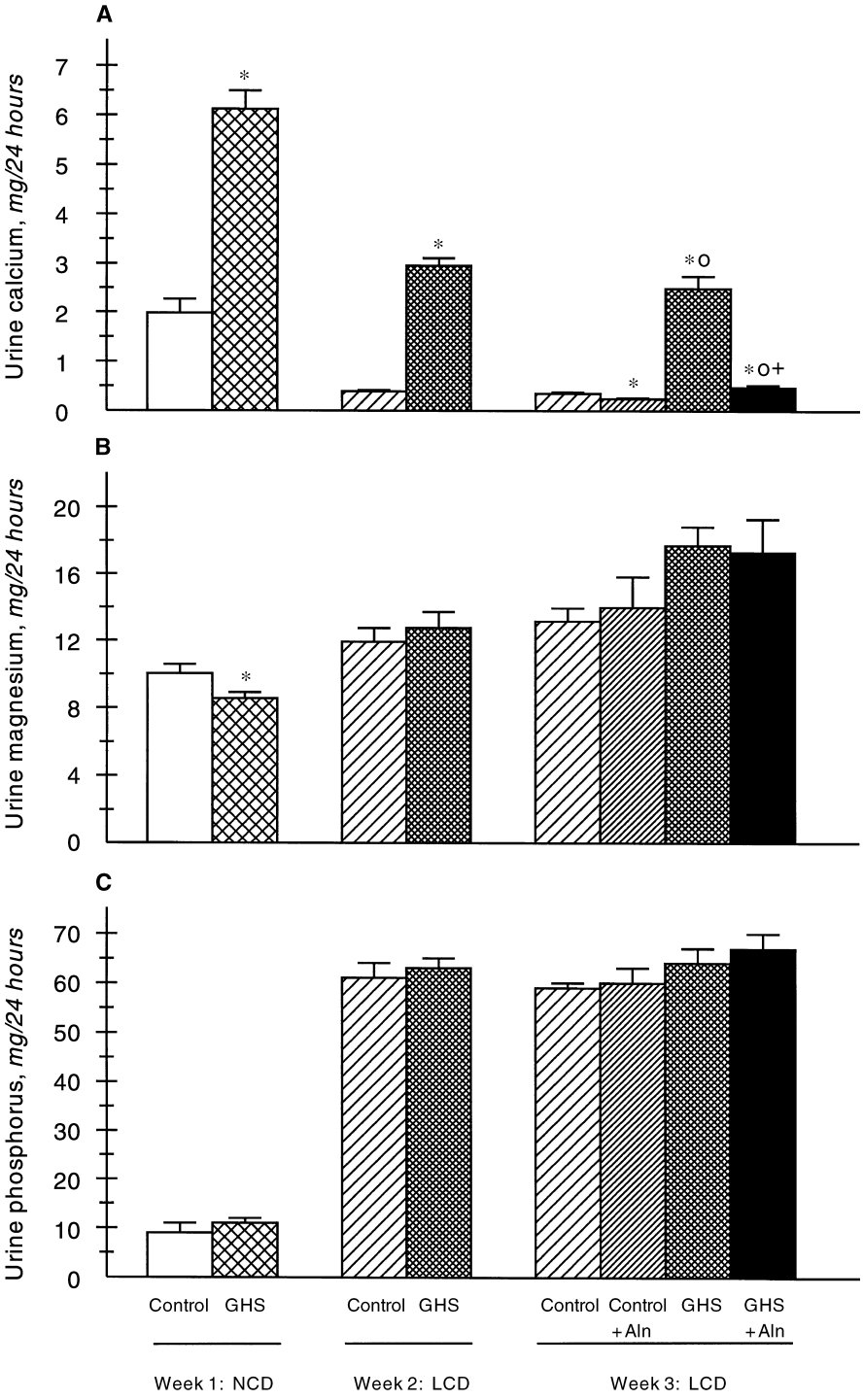

Fig. 2. Urine supersaturation study. Urine calcium, magnesium and phosphorus excretion in genetic hypercalciuric stone forming and control rats. During all three weeks, U_{Ca} of GHS rats exceeded that of Ctl rats (N = 16 during weeks 1 and 2 and N = 8 during week 3). Aln decreased U_{Ca} in both Ctl and GHS rats. Urine magnesium excretion was greater in Ctl than in GHS rats only during week one and was not affected by Aln. During the first week there was no difference in phosphorus excretion between the Ctl and GHS rats and urine phosphorus excretion increased in all groups with LCD and was not affected by Aln. Abbreviations: Ctl, control rats; GHS, genetic hypercalciuric stone forming rats; NCD, normal calcium diet (1.2% calcium); LCD, low calcium diet (0.02% calcium); Aln, (alendronate, ≈ 50 g/kg/24 hr); $^{*}P < 0.05$ vs. same week Ctl; $^{o}P < 0.05$ vs. Ctl + Aln; $^{+}P < 0.05$ vs. same week GHS.

Although this experiment localizes bone as the source of the additional urinary calcium, it does not help to discriminate definitively as to whether the persistence of the IH in the GHS rats on a LCD is from a primary defect in renal tubular calcium resorption or from a primary increase in bone calcium resorption. The reason for this continued uncertainty is that clearance studies were used to show that at the same filtered load of calcium, the GHS rats excrete far more calcium than Ctls, indicating diminished renal calcium reabsorption in these rats [9]. On a LCD, the defect in renal calcium reabsorption would lead to a subtle fall in total and ionized calcium, which would result in an increase in PTH and $1,25(OH)_2D_3$ [4, 41]. The increased PTH and $1,25(OH)_2D_3$ would stimulate

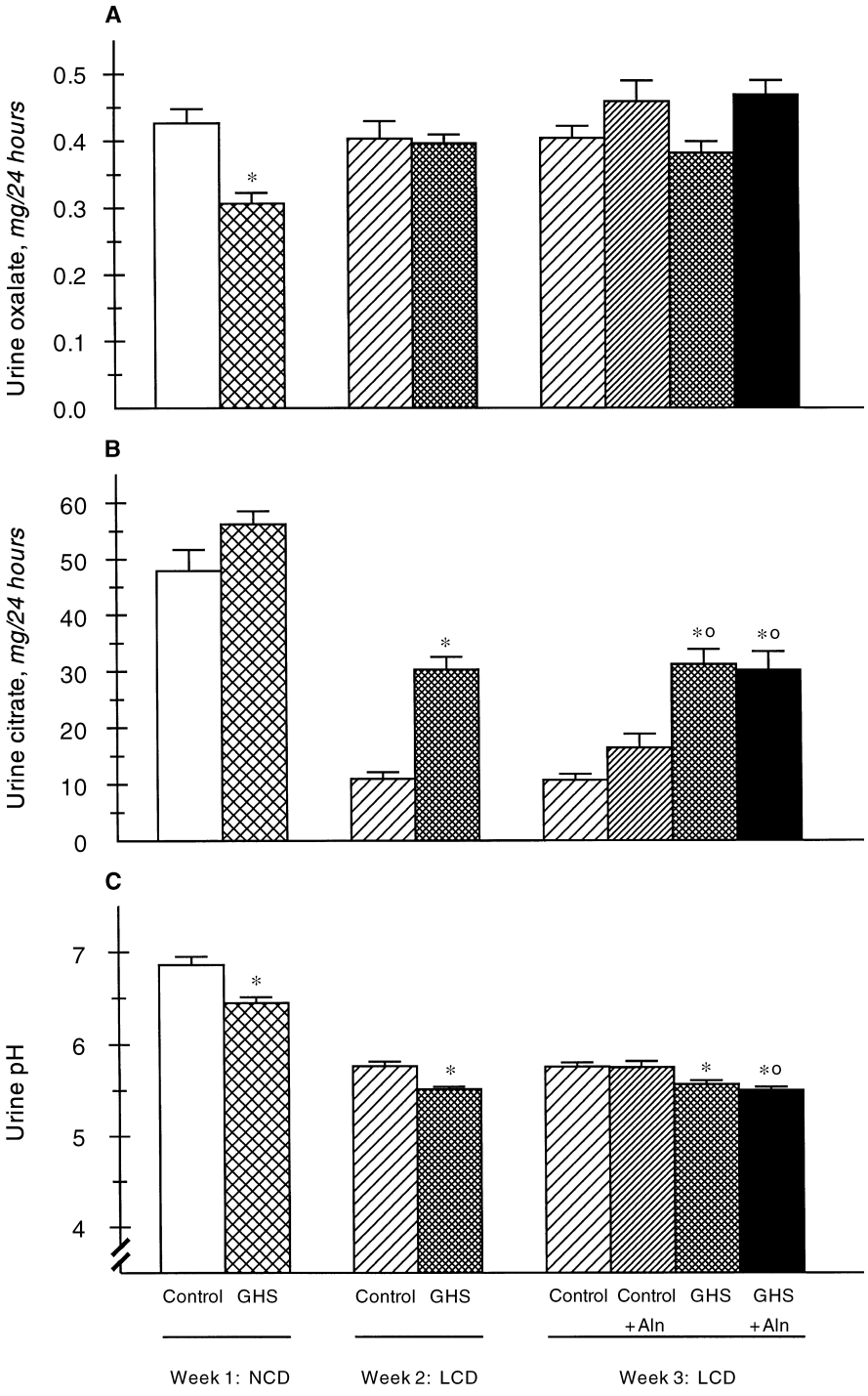

Fig. 3. Urine supersaturation study. Urine oxalate and citrate excretion and pH in genetic hypercalciuric stone forming and control rats. Urine oxalate excretion was greater in the Ctl than in the GHS rats only during week one and urine oxalate excretion was not affected by Aln. During week one there was no difference in urine citrate excretion between the GHS and the Ctl rats; however, urine citrate excretion was greater in each group of GHS, compared to each group of Ctl, rats during week two and during week three. Aln did not affect urine citrate excretion. During the entire study, the urine pH of each group of GHS rats was less than the urine pH of each group of Ctl rats during comparable weeks. Urine pH fell with Aln in the GHS rats. Abbreviations are: Ctl, control rats; GHS, genetic hypercalciuric stone forming rats; NCD, normal calcium diet (1.2% calcium); LCD, low calcium diet (0.02% calcium); Aln, alendronate ($\approx$ 50 g/kg/24 hr); $^{*}P < 0.05$ vs. same week Ctl; $^{o}P < 0.05$ vs. Ctl + Aln.

bone resorption leading to maintenance of the IH at the expense of the bone mineral. Alternatively, the bone from the GHS rats could be undergoing direct enhanced resorption leading to maintenance of the IH, again at the expense of the bone mineral content. This latter mechanism would result in a fall in PTH and $1,25(OH)_2D_3$ [4, 41]. In either case, the bone-resorption blocker Aln would decrease U_{Ca} excretion.

Previously, we have measured serum calcium and $1,25(OH)_2D_3$ in the GHS rats fed a LCD [14]. When fed a LCD, there was no difference in serum calcium between the Ctl and hypercalciuric rats. However, we found that in both the Ctl and GHS rats, serum $1,25(OH)_2D_3$ increased in response to the LCD; however, $1,25(OH)_2D_3$ in the GHS rats did not increase to the same extent as the Ctls. In spite of a lower $1,25(OH)_2D_3$ in the GHS rats,

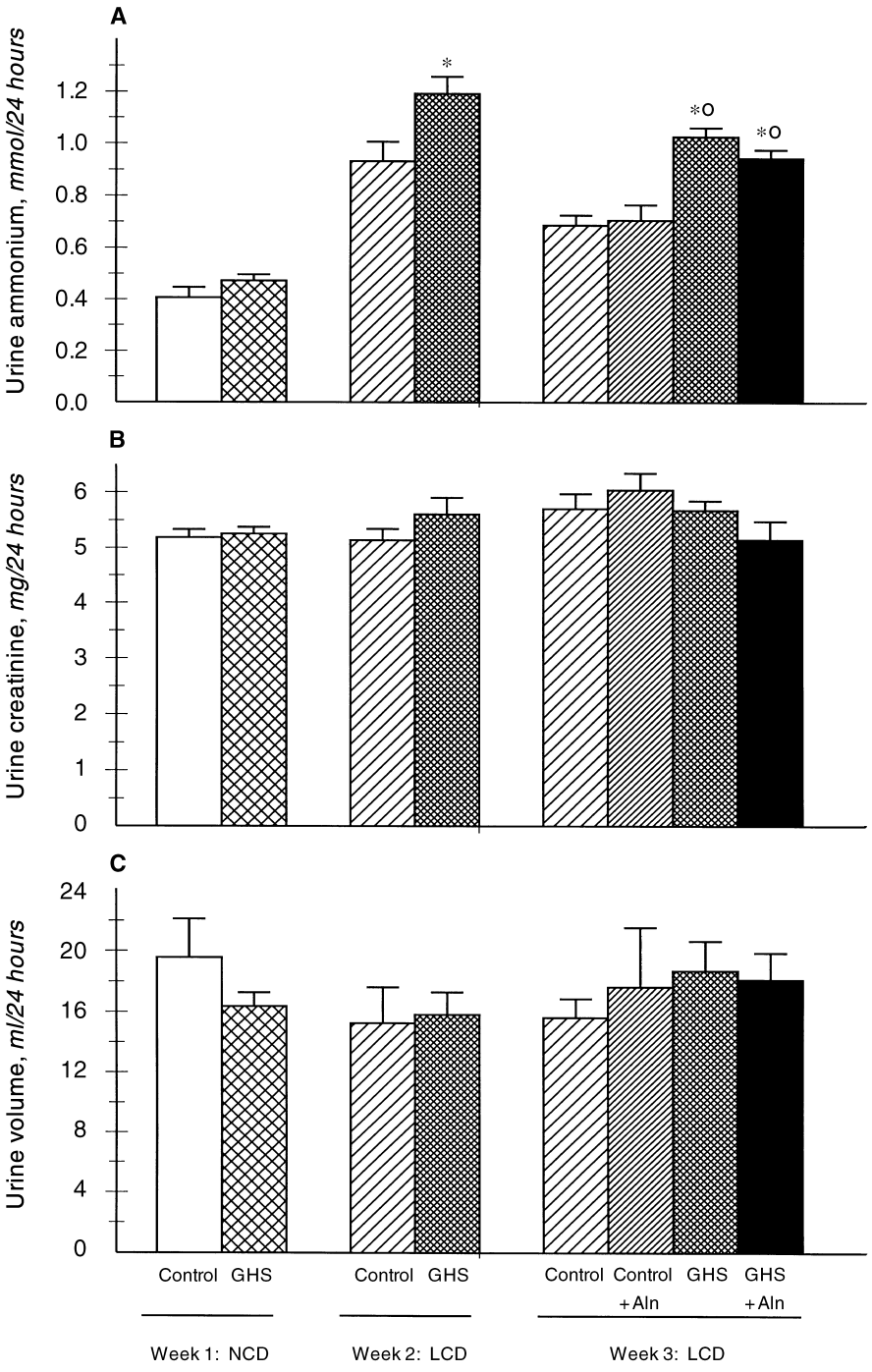

Fig. 4. Urine supersaturation study. Urine ammonium and creatinine excretion and volume in genetic hypercalciuric stone forming and control rats. During week one there was no difference in urine ammonium excretion between the GHS and the Ctl rats; however, urine ammonium excretion was greater in each group of GHS, compared to each group of Ctl, rats during week two and during week three. Aln did not affect urine ammonium excretion. During the entire study, there was no difference in urine creatinine excretion or volume in any group of rats. Abbreviations are: Ctl, control rats; GHS, genetic hypercalciuric stone forming rats; NCD, normal calcium diet (1.2% calcium); LCD, low calcium diet (0.02% calcium); Aln, alendronate, $\approx$ 50 g/kg/24 hr. $^{*}P < 0.05$ vs. same week Ctl; $^{o}P < 0.05$ vs. Ctl + Aln.

their intestinal calcium transport increased to a greater extent. We have previously found that the intestine of the GHS rat has an increased number of vitamin D receptors compared with similarly treated Ctl rats [15]. Perhaps the greater receptor number led to a marked increase in the effect of $1,25(OH)_2D_3$ on intestinal transport in the GHS rats. In addition, we have recently shown that $1,25(OH)_2D_3$ induces a greater up-regulation of vitamin D receptor expression in GHS compared with Ctl rats [8]. Further studies using the GHS rats will be necessary to dissect the regulatory pathways that are responsible for the IH and its persistence when rats are fed a LCD.

The marked fall in the supersaturation of both CaOx and calcium hydrogen phosphate with Aln in the GHS rats would be expected to result in a decrease in the rate and magnitude of stone formation in these rats [7, 10, 12]. We did not examine stone formation in this short-term study, as we have previously shown that after six

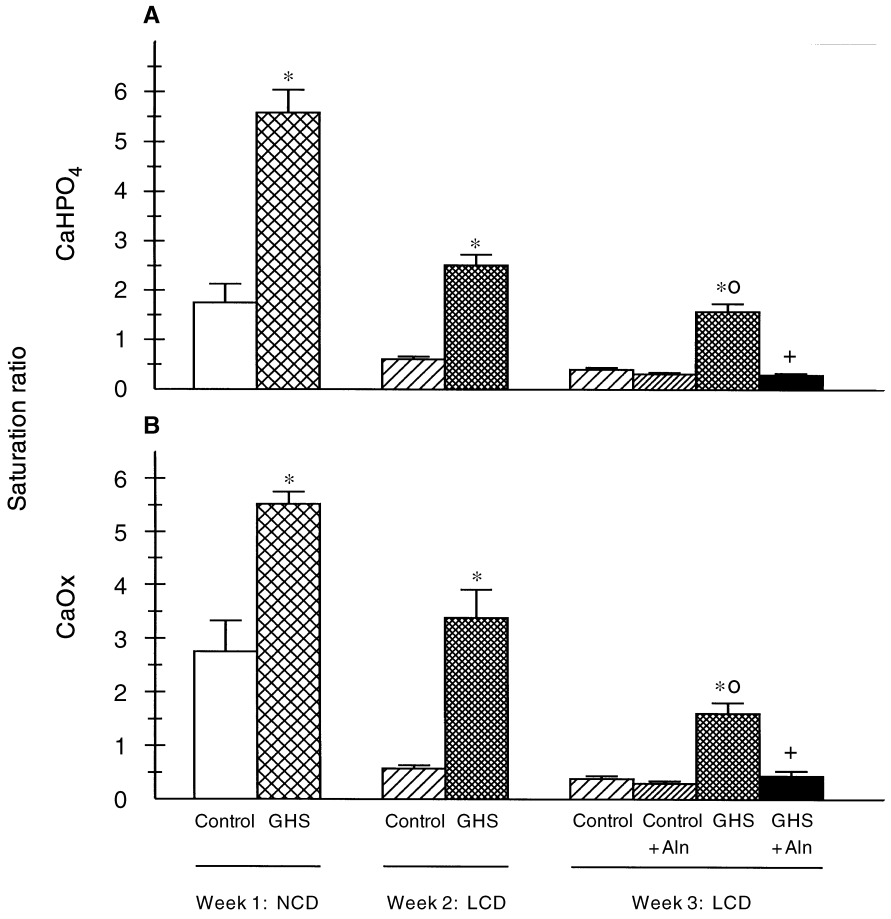

Fig. 5. Urine supersaturation study. Brushite and calcium oxalate supersaturation. During the entire study urine supersaturation with respect to both brushite ($CaHPO_4$) and calcium oxalate (CaOx) were both greater in the GHS rats than in Ctl rats. Aln led to a marked decrease in both brushite and calcium oxalate supersaturation in the GHS, but not in the Ctl, rats. With Aln treatment supersaturation with respect to both brushite and calcium oxalate was decreased such that supersaturation was not different from that of Ctl rats, with or without Aln. Abbreviations are: Ctl, control rats; GHS, genetic hypercalciuric stone forming rats; NCD, normal calcium diet (1.2% calcium); LCD, low calcium diet (0.02% calcium); Aln, alendronate $\approx$ 50 g/kg/24 hr; $^{*}P < 0.05$ vs. same week Ctl; $^{o}P < 0.05$ vs. Ctl + Aln; $^{+}P < 0.05$ vs. same week GHS.

weeks, only one of six GHS rats has demonstrable stone formation and that 18 weeks are required for all GHS rats to form stones [12]. Further long-term studies, perhaps using a longer acting bisphosphonate, will be required to determine if bone-resorption blockade will decrease the universal stone formation in these rats.

In both experiments reported here, the U_{Ca} excretion in the GHS rats fell significantly with Aln, but to a level that remained greater than that of Ctl rats, with or without Aln (Figs. 1 and 2). This observation suggests that the increased intestinal calcium absorption in the GHS rats [10, 14–16] in conjunction with their decreased renal tubular calcium reabsorption [9] allowed some degree of IH to persist, as U_{Ca} excretion was now below the level of dietary calcium intake (2.6 mg/24 hr). Alternatively, there may have been a persistence of bone resorption in spite of the substantial dose of Aln or perhaps there was additional physicochemical bone mineral dissolution. Careful pathological studies of the bone from GHS rats treated with long-term bisphosphonates will help to determine why the IH persisted during Aln treatment.

In this study, the urine phosphorus excretion in both the GHS and the Ctl rats increased when the rats were placed on the LCD. This may be due to the decrease in dietary calcium resulting in less binding of intestinal phosphate, allowing more phosphate to be absorbed and subsequently excreted [42] or as a consequence of the LCD-induced increase in $1,25(OH)_2D_3$-stimulating intestinal phosphorus absorption [41]. As Aln decreases U_{Ca} excretion in the GHS rats fed a LCD, it may have been expected to decrease urine phosphorus as well because bone is composed of calcium phosphorus crystals. However, the magnitude of urine phosphorus excretion is far greater than U_{Ca} excretion (Fig. 2) so that a difference, if present, would be difficult to observe. It is unclear why the urine pH is slightly, but significantly, decreased in the GHS rats compared with the Ctl rats on all diets and why urinary citrate excretion is increased in the GHS rats fed a LCD. The GHS and Ctl rats consumed the same amount of the same diet so that endogenous acid production should be similar in both groups of animals. More acidic urine in the GHS rats may have resulted from an increased ability to excrete acid resulting in relative alkalosis in the Ctl rats and higher citrate excretion [2]. Further studies, perhaps using clearance and/or

microperfusion techniques to study acid transport, will be necessary to determine if there are differences in tubular acid secretion between the GHS and the Ctl rats.

The decrease in U_{Ca} with Aln is probably not due to alterations in renal calcium handling, as the bisphosphonates appear to inhibit bone resorption specifically [20, 21]. In addition, there was far less of a decrease in renal calcium excretion with Aln in the Ctl rats compared with the GHS rats, which would not be the case if Aln increased renal tubular calcium reabsorption. However, the effects of Aln, or other bisphosphonates, on renal calcium excretion have not been reported using formal clearance or perfusion techniques to completely exclude an effect on renal calcium reabsorption.

The reduction in calcium from the 1.2% calcium diet to the 0.02% calcium diet was achieved, in large part, by a decrease in the calcium carbonate content of the diet. This decrease in dietary base probably led to the decrease in urinary pH (Fig. 3) and the increase in urinary ammonium (Fig. 4) of both the Ctl and GHS rats fed the LCD. The change in dietary base content could not have been responsible for the effects of Aln on bone, as all rats receiving Aln were being fed the LCD.

The GHS rats are analogous to humans with idiopathic IH in many respects. In humans, idiopathic IH appears to be genetic [2, 43–46]. Many patients exhibit an increase in intestinal calcium absorption and a decrease in renal tubular calcium resorption [44, 47–50]. Our inbred rat model of IH appears analogous to humans in these respects [10]. In humans, there is evidence for a decrease in bone mineral density in patients with idiopathic IH, suggesting that bone resorption accounts, at least in part, for human IH [51–55]. An Aln-induced decrease in urine supersaturation should be beneficial in preventing stone formation in humans, if these results, observed in a short-term study using the hypercalciuric stone-forming rat, can be confirmed in long-term human studies.

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APPENDIX

Abbreviations used in this article are: Aln, alendronate; CaOx, calcium oxalate; Ctl, control; GHS, genetic hypercalciuric stone forming; IH, idiopathic hypercalciuria; LCD, low-salt diet; NCD, normal calcium diet; U_{Ca}, urinary calcium.

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